

Effectiveness of Irradiation Treatments in Inactivating *Listeria monocytogenes* on Fresh Vegetables at Refrigeration Temperature[†]

M. L. BARI,^{1*} M. NAKAUMA,² S. TODORIKI,² VIJAY K. JUNEJA,³ K. ISSHIKI,¹ AND S. KAWAMOTO¹

¹Food Hygiene Team and ²Radiation and Information Technology Laboratory, National Food Research Institute, Kannondai-2-1-12, Tsukuba 305-8642, Japan; and ³U.S. Department of Agriculture, Agricultural Research Service, Wyndmoor, Pennsylvania 19038, USA

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ABSTRACT

Ionizing radiation can be effective in controlling the growth of food spoilage and foodborne pathogenic bacteria. This study reports on an investigation of the effectiveness of irradiation treatment to eliminate *Listeria monocytogenes* on laboratory-inoculated broccoli, cabbage, tomatoes, and mung bean sprouts. Irradiation of broccoli and mung bean sprouts at 1.0 kGy resulted in reductions of approximately 4.88 and 4.57 log CFU/g, respectively, of a five-strain cocktail of *L. monocytogenes*. Reductions of approximately 5.25 and 4.14 log CFU/g were found with cabbage and tomato, respectively, at a similar dose. The appearance, color, texture, taste, and overall acceptability did not undergo significant changes after 7 days of postirradiation storage at 4°C, in comparison with control samples. Therefore, low-dose ionizing radiation treatment could be an effective method for eliminating *L. monocytogenes* on fresh and fresh-cut produce.

Listeria monocytogenes has been associated with a number of serious foodborne outbreaks and product recalls (1, 11). The bacterium is a psychrotroph and can survive and grow at temperatures as low as 4°C; thus, it can be a significant problem in refrigerated foods (9, 16, 33). Contamination with *L. monocytogenes* has been primarily associated with the consumption of dairy products, beef, pork, poultry, and seafood. However, an increasing body of data supports and suggests that salad vegetables, such as cabbage, celery, lettuce, cucumber, onion, leeks, watercress, radish, tomatoes, and fennel, among others, can have a high incidence of *L. monocytogenes*, and some of these products have been implicated in outbreaks of foodborne listeriosis (4, 6, 24, 31). Outbreaks of listeriosis usually occur at pathogen populations greater than 10³ CFU per g or per ml (32). The *L. monocytogenes* serotypes 1/2a, 1/2b, and 4b are of particular concern since they account for up to 96% of human listeriosis cases throughout the world (32). Because of the high case fatality rate associated with *L. monocytogenes* infections, the U.S. Food and Drug Administration and the U.S. Department of Agriculture, Food Safety and Inspection Service have established a zero tolerance policy (no detectable level permitted) for *L. monocytogenes* in ready-to-eat foods, including minimally processed fresh and fresh-cut fruits and vegetables. Several food recalls have been triggered by possible *L. monocytogenes* contamination during the last few years, including a recall of packaged fresh-cut apples (23 to 27 March 2001; see the U.S. Food and

Drug Administration web site at http://www.fda.gov/oc/po/firmrecalls/FreshProd3_01.html).

With the increasing consumption of fresh produce and increased globalization of the fresh produce market, the presence of *L. monocytogenes* is of increasing concern in produce because of its likely association with foodborne illness (4, 28–30). Conventional antimicrobial procedures such as washing, chemical sanitization, thermal treatment, and modified atmosphere packaging have historically been developed and refined to effectively suppress spoilage organisms (19). Much research is dedicated to improving the efficacy of intervention technologies against pathogenic bacteria such as *L. monocytogenes*, *Salmonella*, and *Escherichia coli* (5, 17, 18). However, it is increasingly recognized that leaves, fruits, and seeds provide bacteria with numerous mechanisms to counter these antimicrobial measures. As recent research has shown, bacteria not only are likely to enter fruits and vegetables through natural openings (stomata, calyx, stem, stem scar, etc.), abiotic wounds, or damage caused by phytopathogens, but they also can survive within the produce for days or weeks (2). The consequences of internalization are reduction in the effectiveness of chemical treatments or any other applied intervention (5, 14, 35).

Ionizing radiation can effectively eliminate *L. monocytogenes* on processed meat products (25) and a variety of fresh and frozen products of animal origin (12). Ionizing radiation is able to penetrate into protected areas of produce (surface, subsurface, and interior) to injure or destroy bacteria; however, the extent to which a biofilm habitat may influence the radiation sensitivity of bacteria, either native nonpathogens or pathogenic contaminants, is not well understood. The purpose of this study was to determine the

* Author for correspondence. Tel: +81-29-838-8067; Fax: +81-29-838-8067; E-mail: latiful@nfri.affrc.go.jp.

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effectiveness of irradiation treatment in eliminating *L. monocytogenes* in fresh-cut broccoli and cabbage and whole tomatoes and mung bean sprouts.

MATERIALS AND METHODS

Test strains. *L. monocytogenes* strains ATCC 43256 (from Mexican-style cheese), ATCC 49594 (derived from *L. monocytogenes* strain Scott A; American Type Culture Collection, Manassas, VA), JCM 7676 (from roast beef), JCM 7672 (from salami sausage), and JCM 7671 (from lax ham; Japan Collection of Microorganisms) were used as challenge organisms in this study. To minimize the growth of microorganisms naturally present on fresh produce, all test strains of *L. monocytogenes* were grown in tryptose phosphate broth (pH 7.0; Difco, Becton Dickinson, Sparks, Md.) containing 50 µg of nalidixic acid per ml before their use as an inoculum. Plating on media containing nalidixic acid minimized interference with colony development by naturally occurring microorganisms, thus facilitating detection of the test pathogen on recovery media.

Preparation of inocula. Each strain of *L. monocytogenes* was cultured in tryptose phosphate broth containing 50 µg of nalidixic acid per ml at 37°C at three successive 24-h intervals immediately before use as inocula. Cells of each strain were collected by centrifugation (3,000 rpm, 10 min, 20°C) and resuspended in 5 ml of phosphate-buffered saline (pH 7.2). Equal volumes of cell suspensions of five strains were combined to give approximately equal populations of each strain. The inoculum was maintained at 21 ± 1°C and applied to the test vegetables within 1 h of preparation.

Inoculation procedure. Commercial cabbage, mung bean sprouts, broccoli, and tomato used in these experiments were purchased from a local supermarket and stored at refrigerated temperature (4°C) for a period of 4 to 6 h before being used in the experiments. Before inoculation, the commercial packages of mung bean sprouts and tomatoes were opened aseptically and the produce washed with sterile distilled water. Cabbage and broccoli were also washed with sterile distilled water and cut into pieces using a sterile cutting board and knife. Five hundred grams of each cut produce, and mung bean sprouts were dipped separately in each 2-liter cocktail cell suspension (ca. 10⁸ CFU/ml) for 1 min. Forty-eight whole tomatoes (for one set) were dipped in 2 liters of cocktail cell suspension (ca. 10⁸ CFU/ml) for 1 min. After the inoculum was decanted, the produce was placed separately on crystalline dishes in a biosafety cabinet to dry for 1 to 2 h. One hundred grams of each cut vegetable, 100 g of mung bean sprouts, and two or three tomatoes were put into sterile Ziploc plastic bags (Seinichi Co. Ltd., Tokyo, Japan) and were irradiated within 8 h.

Irradiation. The inoculated samples were treated with 0.0 (control), 0.2, 0.4, 0.6, 0.8, and 1.0 kGy radiation doses. For each vegetable, the experiment was performed three times in separate trials using separate sets of vegetable samples. All irradiation experiments on a given day were conducted at a single temperature to maintain consistency in temperature control. The samples were irradiated at a dose rate of 1.4 kGy/h from a cobalt-60 gamma source of 16,841 Ci (Nordion International Inc., Kanata, Ontario, Canada; Gamma Cell-220). The absorbed dose was confirmed with a cellulose triacetate film dosimeter (FTR-125, Fuji Photo Film Co. Ltd., Tokyo, Japan), which was attached to the surface of the plastic bag, according to McLaughlin et al. (20). After irradiation, samples were kept at 4°C for 10 days and their microbiological and sensory parameters were evaluated at 1, 3, 5, 7, and 10 days.

Microbiological analyses. One hundred grams of irradiated and nonirradiated samples were aseptically transferred to Stomacher bags, and 100 ml of 0.1% peptone water was added. The bag contents were pummeled for 60 s in a stomacher (ILU Instrument, model CE-97, Barcelona, Spain) at medium speed. Serial decimal dilutions were prepared with 0.1% peptone water, and the diluted and undiluted samples were surface plated (0.1 ml, in duplicate) on tryptose phosphate agar for total bacterial counts, tryptose phosphate agar supplemented with 50 µg/ml nalidixic acid (TPAN), and modified Oxford medium (Oxoid) supplemented with 50 µg/ml nalidixic acid (MOXN) for *L. monocytogenes*. Inoculated enumeration media were incubated at 37°C for 24 to 48 h before presumptive colonies of each pathogen were counted. At least five randomly picked presumptive colonies of *L. monocytogenes* were confirmed with API *Listeria* diagnostic kits (bioMérieux, Durham, N.C.). The total viable bacterial count and total coliform count were determined on standard plate count agar (Nissui) and desoxycholate agar (Nissui), respectively. The total fungal count and *Salmonella* count was done using potato dextrose agar (Difco) and S-S agar (Difco), respectively. The inoculated enumeration media were incubated at 37°C for 24 to 48 h for total viable bacteria, coliform, and *Salmonella* count. However, total fungal counts were obtained after incubating the plates at 30°C for 3 to 5 days.

Sensory evaluation. The quality of irradiated and nonirradiated noninoculated fresh produce was evaluated by panelists (10 judges) selected from different departments of the National Food Research Institute who were experienced with sensory panels. Evaluation was conducted in a properly equipped taste panel booth. The evaluation characteristics of appearance, color, taste, and overall acceptability was based on a nine-point hedonic scale: 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither dislike nor like, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely. For texture, the hedonic scale was 1 = extremely hard, 2 = very hard, 3 = moderately hard, 4 = slightly hard, 5 = neither hard nor soft, 6 = slightly soft, 7 = moderately soft, 8 = very soft, 9 = extremely soft.

The sensory characteristics of appearance, texture, color, taste, and overall acceptability were assessed on each test sample. For each of the quality attributes, three packs of each irradiated and nonirradiated produce were examined or tested or both by the judges. Rejection of sample was based on a score of 5.0, which was the quality criterion used for both irradiated and nonirradiated fresh produce. For the taste evaluation of broccoli and mung bean sprouts, the samples were heated in a microwave for 2 and 1 min, respectively, before taste evaluation.

Statistical analyses. All trials were replicated three times. Reported plate count data represent the mean values obtained from three individual trials, each of these values being obtained from duplicated samples. Significant differences in plate count data were established by least significant difference at the 5% level of significance.

RESULTS AND DISCUSSION

The total initial bacterial load of broccoli and cabbage was recorded as 3.21 and 4.15 log CFU/g, respectively. The total initial bacterial count of tomato and mung bean sprouts was 2.85 and 5.17 log CFU/g, respectively (Table 1). These results reveal that the total bacterial count of cabbage is higher than that of broccoli. Coliform were found in broccoli, cabbage, and mung bean sprouts, and the total

TABLE 1. *Bacterial populations of broccoli, cabbage, tomato, and mung bean sprouts before and after irradiation*

	Populations (log CFU/g) ^a			
	Broccoli	Cabbage	Tomato	Bean sprout
Control				
Tryptic soy agar	3.21	4.15	2.85	5.17
Desoxycholate agar	0.86	1.01	<1.0 ^b	1.87
S-S agar	<1.0 ^b	<1.0 ^b	<1.0 ^b	<1.0 ^b
Potato dextrose agar	<1.0 ^b	<1.0 ^b	<1.0 ^b	<1.0 ^b
After treatment ^c				
0.2 kGy	2.25	3.06	1.36	3.55
0.4 kGy	1.20	1.97	0.66	2.26
0.6 kGy	0.68	0.86	0.17	1.03
0.8 kGy	0.23	0.14	<1.0 ^b	0.17
1.0 kGy	<1.0 ^b	<1.0 ^b	<1.0 ^b	<1.0 ^b

^a Mean values of three replicate experiments ($P \leq 0.05$).
^b No colonies were observed.
^c Populations recovered on tryptic soy agar medium.

coliform count was recorded as 0.86, 1.01, and 1.87 log CFU/g, respectively. However, no coliform were found on tomatoes. The presence of *Salmonella* in fresh produce is of special significance because of the public health importance of this organism. However, no *Salmonella* or fungi were detected in any vegetable sample tested. The high microbial load observed on mung bean sprouts in comparison with other vegetables indicates poor hygienic conditions during processing and storage at improper temperatures. Previous studies (3, 10, 27) have shown that traditional selective media may not support colony development of *L. monocytogenes* exposed to some of the antimicrobial treatments evaluated in this study. Although nalidixic acid may

prevent the growth of the majority of microorganisms that might be present on fresh produce, the potential for interference by background microbiota of *L. monocytogenes* on TPAN exists. For these reasons, TPAN and MOXN were used in all experiments for enumeration of *L. monocytogenes* on treated and untreated fresh produce. In the present study, *L. monocytogenes* were not recovered from uninoculated fresh produce. Regardless of fresh produce conditions or treatments, higher populations of *L. monocytogenes* were recovered on TPAN than on MOXN. *L. monocytogenes* counts were 0.10 to 0.60 log CFU/g higher when samples from inoculated fresh produce washed with water (control) were plated on TPAN compared with selective medium. Larger differences between bacterial counts on TPAN and selective media were observed in fresh produce treated with different radiation doses. This is in agreement with other reports describing the poor performance of selective medium in recovering pathogens from treated fresh produce (7). Lower counts on highly selective media may be due to the inability of cells that have been injured by desiccation or treatment to resuscitate.

Listed in Table 2 are populations of *L. monocytogenes* on broccoli and mung bean sprouts with or without exposure to different doses of radiation. Irrespective of the vegetable, populations of surviving bacteria gradually decreased with the increased dose of radiation, and at 1.0 kGy irradiation eliminated the pathogen completely in both broccoli and mung bean sprouts. The radiation dose necessary to reduce the bacterial population by 90% (D_{10} -values) for *L. monocytogenes* differed significantly among vegetables. The average D_{10} -values for *L. monocytogenes* in broccoli and mung bean sprouts were 0.20 and 0.22 kGy, respectively.

Populations of *L. monocytogenes* on cabbage and to

TABLE 2. *Recovery of Listeria monocytogenes from inoculated broccoli and mung bean sprouts after irradiation treatment and storage for 10 days at 4°C*

Irradiation dose	Populations recovered (CFU/g) ^a											
	1-day reduction			3-day reduction			7-day reduction			10-day reduction		
	MXON	TPAN	(CFU/g)	MXON	TPAN	(CFU/g)	MXON	TPAN	(CFU/g)	MXON	TPAN	(CFU/g)
Broccoli												
Control	4.63	4.88	—	4.36	4.55	—	4.10	4.30	—	3.95	4.15	—
0.2 kGy	3.51	3.64	1.24	3.27	3.47	1.08	2.97	3.26	1.04	2.70	3.05	1.10
0.4 kGy	2.35	2.57	2.31	2.56	2.78	1.77	1.73	1.94	2.36	1.84	2.04	2.11
0.6 kGy	1.14	1.34	3.54	1.31	1.53	3.02	0.92	1.02	3.28	0.76	0.97	3.18
0.8 kGy	0.12	0.41	4.47	0.24	0.44	4.11	<1.0 ^b	0.11 ^b	4.29	<1.0 ^b	0.10	4.05
1.0 kGy	<1.0 ^b	<1.0 ^b	4.88	<1.0 ^b	<1.0 ^b	4.55	<1.0 ^b	<1.0 ^b	4.30	<1.0 ^b	<1.0 ^b	4.15
Mung bean sprouts												
Control	4.48	4.57	—	4.54	4.65	—	4.61	4.79	—	4.50	4.66	—
0.2 kGy	3.28	3.39	1.18	3.12	3.34	1.31	3.33	3.69	1.10	3.09	3.29	1.37
0.4 kGy	2.07	2.21	2.36	2.03	2.20	2.45	2.47	2.60	2.19	1.98	2.11	2.55
0.6 kGy	1.01	1.19	3.38	0.97	1.12	3.53	1.22	1.33	3.46	0.47	0.94	3.72
0.8 kGy	0.29	0.45	4.12	<1.0 ^b	0.23	4.42	0.12	0.31	4.48	<1.0 ^b	0.10	4.56
1.0 kGy	<1.0 ^b	<1.0 ^b	4.57	<1.0 ^b	<1.0 ^b	4.65	<1.0 ^b	<1.0 ^b	4.79	<1.0 ^b	<1.0 ^b	4.66

^a Mean values of three replicate experiments ($P \leq 0.05$).
^b No colonies were observed.

TABLE 3. Recovery of *Listeria monocytogenes* from inoculated cabbage and tomato after irradiation treatment and storage for 10 days at 4°C

Irradiation dose	Populations recovered (CFU/g) ^a											
	1-day reduction			3-day reduction			7-day reduction			10-day reduction		
	MXON	TPAN	(CFU/g)	MXON	TPAN	(CFU/g)	MXON	TPAN	(CFU/g)	MXON	TPAN	(CFU/g)
Cabbage												
Control	5.13	5.25	—	4.96	5.04	—	4.70	4.76	—	4.60	4.66	—
0.2 kGy	4.00	4.04	1.21	3.67	3.97	1.07	3.87	3.96	0.80	3.90	3.95	0.71
0.4 kGy	2.72	2.97	2.28	2.56	2.78	2.26	2.43	2.84	1.92	2.34	2.44	2.22
0.6 kGy	1.47	1.77	3.48	1.31	1.53	3.51	0.72	0.82	3.94	0.45	0.65	4.01
0.8 kGy	1.05	1.12	4.13	0.24	0.44	4.60	<1.0 ^b	0.21	4.55	<1.0 ^b	<1.0 ^b	4.66
1.0 kGy	<1.0 ^b	<1.0 ^b	5.25	<1.0 ^b	<1.0 ^b	5.04	<1.0 ^b	<1.0 ^b	4.76	<1.0 ^b	<1.0 ^b	4.66
Tomato												
Control	3.87	4.14	—	3.04	3.44	—	2.96	3.13	—	2.50	2.76	—
0.2 kGy	2.93	3.12	1.02	2.07	2.27	1.17	2.03	2.17	0.96	1.92	2.04	0.72
0.4 kGy	1.78	1.81	2.33	1.06	1.18	2.26	1.09	1.21	1.92	0.98	1.13	1.63
0.6 kGy	0.91	1.16	3.48	0.21	0.43	3.01	0.22	0.31	2.82	0.17	0.23	2.53
0.8 kGy	<1.0 ^b	0.12	4.02	<1.0 ^b	<1.0 ^b	3.44	<1.0 ^b	<1.0 ^b	3.13	<1.0 ^b	<1.0 ^b	2.76
1.0 kGy	<1.0 ^b	<1.0 ^b	4.14	<1.0 ^b	<1.0 ^b	3.44	<1.0 ^b	<1.0 ^b	3.13	<1.0 ^b	<1.0 ^b	2.76

^a Mean values of three replicate experiments ($P \leq 0.05$).

^b No colonies were observed.

mato with or without exposure to different doses of radiation are listed in Table 3. Regardless of the vegetable, populations gradually decreased with the increased dose of radiation, and at 1.0 kGy irradiation eliminated the pathogen completely in both cabbage and tomato. The average D_{10} values for *L. monocytogenes* in cabbage and tomato were 0.19 and 0.24 kGy, respectively. However, the populations of *L. monocytogenes* gradually decreased in all the control samples at 10 days storage except for mung bean sprouts (Tables 2 and 3). For mung bean sprouts, populations grad-

ually increased at 3 and 7 days or remained constant throughout the storage period.

In a previous study, a dose of 1.0 kGy reduced the total aerobic plate count and *L. monocytogenes* by approximately 4.0 logs (99.99%) on precut bell pepper; however, on peppers stored at 15 or 10°C, the pathogen regrew to initial levels within 4 days, while pathogen levels remained low on peppers stored at 5°C (13). In the same study, spoilage bacteria were reduced by 5 log cycles on carrot cubes by 1.0 kGy. The authors concluded that irradiation, when

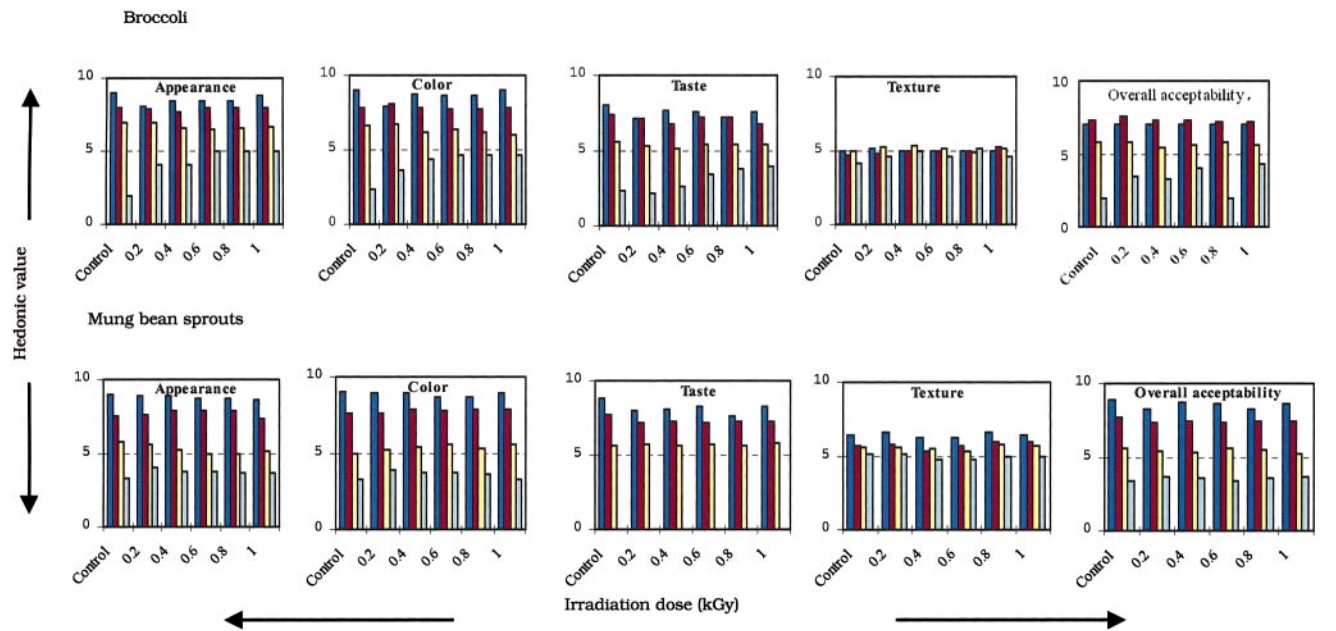


Figure 1. The appearance, color, taste, texture, and overall acceptability of broccoli and mung bean sprouts stored at 4°C for 10 and 7 days, respectively, following different irradiation doses. Broccoli: ■, 1 day, ■, 3 days, ■, 7 days, ■, 10 days. Mung bean sprouts: ■, 1 day, ■, 3 days, ■, 5 days, ■, 7 days.

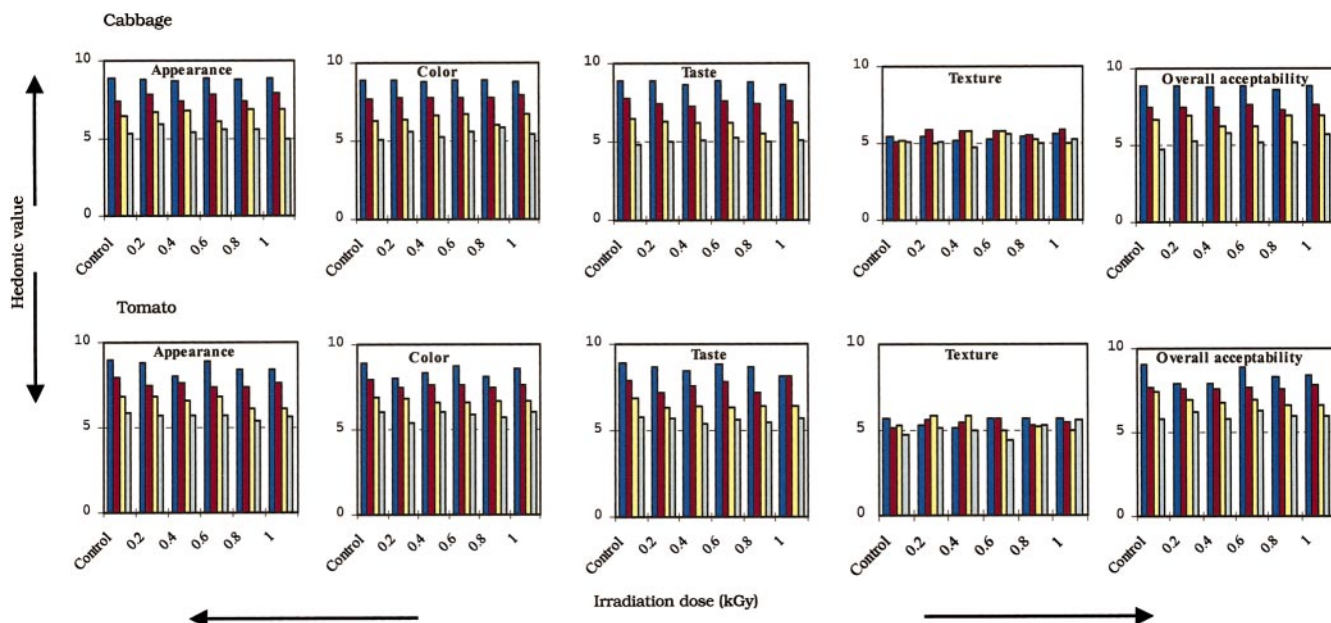


Figure 2. The appearance, color, taste, texture, and overall acceptability of cabbage and tomato stored at 4°C for 10 and 7 days, respectively, following different irradiation doses. Cabbage and tomato: ■, 1 day, ■, 3 days, ■, 7 days, ■, 10 days.

combined with good manufacturing practices, could effectively reduce pathogen levels throughout the useful shelf life of the produce. In our study, we also found that a dose of 1.0 kGy could reduce total aerobic plate count and *L. monocytogenes* by approximately 4.0 to 5.0 log CFU/g, depending on the types of produce.

Vegetable samples subjected to different radiation doses were given to a group of judges on different days of storage for their assessment of sensory parameters. Figures 1 and 2 depict that the produce treated with a dose of 1.0 kGy could be stored up to 1 week, reaching the borderline of acceptability. For mung bean sprouts, the maximum acceptable storage period was 5 days with 1.0 kGy dose of radiation. The shorter period of storage for mung bean sprouts may be due to the poor hygienic condition prevalent in the commercial shops as well as improper storage conditions and temperatures. In the present study, five parameters chosen as indices of acceptability of treated samples were appearance, color, taste, texture, and overall acceptability limit values for the vegetables tested.

Radiation-induced softening due to hydrolysis of pectin is a well-known phenomenon that is radiation-dose dependent (34). Irradiated (1.0 kGy) celery maintained its quality throughout the 22-day storage period of study (21). Treatments up to 2.0 kGy did not have any negative effect on radish sprouts (22). A dose of 1.0 kGy caused a 12% loss of ascorbic acid content in pre-cut bell pepper and a slight increase in beta-carotene content of cubed carrots, and the dose extended the shelf life of both products (13). Fruits and vegetables present a complex substrate for studies of irradiation, with significant complicating factors of maturity, anatomy, topology, surface and internal chemistry, and native microflora, which may compete with or succor contaminating pathogenic bacteria. By providing microhabitats with a unique microflora or biofilm community or a localized complement of secondary metabolites, plant surfaces

with more varied topography may result in increased survival of pathogens in protective niches. Bacteria are known to penetrate into the tissues of lettuce (26) and apple (8, 15, 18, 23), making them inaccessible to chemical disinfectants. The anatomical structures of most fruits and vegetables do not present a significant barrier to ionizing photons (gamma and X ray), although the depth of penetration of ionizing electrons may be a factor for unusually thick or dense products. The effect of these factors, singly and in combination, will determine what doses will be most effective at eliminating contaminating bacteria and is best tolerated by the fruit and vegetable products of interest.

The use of a number of acceptability parameters is recommended to allow the observation of different treatments on such parameters. In addition, as many parameters as possible should be used to determine consumer acceptability. The best storage period obtained when applying 1.0 kGy was 7 days, taking into account the acceptable limit of each of the parameters independently and keeping all the parameters within acceptable limit. However, for mung bean sprouts, there was at least 5 days storage with 1.0 kGy irradiation. Therefore, further studies are needed to determine preirradiation and postirradiation storage condition and suitability of packaging materials, quality assessment, and optimization of radiation dose. Since irradiation causes reduction of microbial population, application of this technique along with proper packaging conditions may significantly increase the shelf life. Results obtained in the present study will be applied to other varieties of fresh produce consumed in developing countries, leading to microbiologically safer produce with extended shelf life.

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